APOLIPOPROTEIN B AND CORONARY ARTERY DISEASE IN INDIAN POPULATION

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FEBRUARY 2007.

CERTIFICATE

This is to certify that the thesis titled "**APOLIPOPROTEIN B AND CORONARY ARTERY DISEASE IN INDIAN POPULATION"** is the bonafide work of **Dr. Vimalraj B.S.** done towards partial fulfillment towards requirements of the DM Branch II (Cardiology) examination of the Dr. MGR Medical University, Chennai, Tamil Nadu, to be conducted in February 2007.

Guide:

Dr. V. Jacob Jose
MD DM (Card) FACC MS FCCP FIAE
Professor and Head of Unit II,
Department of Cardiology,
Christian Medical College,
Vellore.

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Dr. George Joseph MD DM (Card)
Professor and Head of Cardiology,
Department of Cardiology,
Christian Medical College,
Vellore.

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Dr. Vimalraj B.S.

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ABSTRACT

Aim:

To study the association between plasma apolipoprotein B (apo B) and angiographically proven Coronary Artery Disease (CAD) in a prospective manner.

Background:

The plasma apo B concentration represents the number of atherogenic lipoproteins and it has been demonstrated that it could be a predictor for CAD. This study was done to find out whether apo B is an independent risk factor for CAD and whether apo B is superior to routine lipid profile in differentiating patient with CAD.

Materials and Methods:

The study population consisted of both men and women who were undergoing their first angiography between March 2004 and December 2005. A total of 200 consecutive patients were enrolled. Patients were divided in to 2 groups; Group 1: Patients with angiographically proved CAD were included.

Group 2: Patients with normal coronary angiogram or patients with negative stress testing by treadmill. After an overnight fasting, blood samples were taken for lipids and apo B. Baseline characteristics such as hypertension, diabetes, and smoking were taken from all the patients. A patient was said to have CAD (CAD+) if there was an angiographic lesion more than 50%. A patient was considered as control (CAD-), if angiogram was normal or treadmill stress testing was negative.

Results:

A total of 200 patients were enrolled. There were 155 males and 45 females in the study. There were slightly more number of males in group 1. The total cholesterol was higher in patients with CAD when compared to controls. When we use the cut off value of 150mg or above as hypertriglyceridemia, then 52% of CAD patients were found to have higher values compared to controls, which was only 35%. For a cut off value of LDL less than 130mg%, it was found that 94% of controls and 83% of patients with CAD had lower values.

The median apo B values in patients with CAD were 1.07g/L, versus in controls 0.79g/L, which was statistically significant. We calculated Receiver Operating Curves (ROC) to assess the sensitivity and specificity of apo B in identifying a patient with CAD. For a cut off value 0.99 g/L, it was found that the sensitivity was 66% and specificity was 92%. The area under the curve was 0.814.

Conclusion:

Our results suggest that apolipoprotein B provides better information regarding the presence of CAD. Higher apo B values were noted even in those patients with CAD with normal levels of LDL. In patients who were on statins only apo B was able to predict the presence of CAD. Apo B is a better predictor for CAD than routine LDL levels.

INTRODUCTION

The association between serum cholesterol levels and atherosclerosis in humans was suggested when Thannhauser and Muller in 1938 demonstrated familial aggregation of individuals with tendon xanthomata, hypercholesterolemia, and CAD.[1] The association was generalized by studies such as those in Framingham, which demonstrated that the risk for coronary artery disease rose over the entire range of serum cholesterol. This relation was seen predominantly in those persons 30 to 49 years of age at entry into the study and most markedly in those 30 to 39 years old.

Different lipoproteins affect risk differently. Among subjects 49 years and older, low density lipoprotein (LDL) cholesterol was associated with moderate CAD risk, however, in these older individuals, the high density lipoprotein (HDL) cholesterol level seemed to have greater inverse predictive value.[2] Decreased HDL cholesterol also was shown to be associated with CAD risk in younger people.[3] And finally, elevated triglyceride (TGL) levels have been linked with CAD [4-6] but may not be independent of the effect of other lipoproteins or of obesity.[7]

Alaupovic[8] was the first to suggest that apoproteins should be considered when evaluating lipoprotein disorders, and in the early 1970s several groups [9] demonstrated elevations in plasma LDL and/or very low-density lipoprotein (VLDL) apo B levels in patients with most types of hyperlipidemia.

In 1978 Avogaro et al. [1] found that total plasma apo B levels were elevated in individuals with CAD regardless of whether they were normocholesterolemic or hypercholesterolemic. Vergani et al.[10] and others [11-15] also found higher plasma apo B levels in subjects with atherosclerosis than those apparently free of atherosclerosis. Indeed, higher plasma apo B levels were reported in persons with CAD compared to those without CAD, even in those with normal lipid levels.[16]

Sniderman et al. [1] measured apo B levels in LDL separated from VLDL by exclusion gel radial immunodiffusion or by preparative ultracentrifugation. Among those with CAD diagnosed by angiography with normal LDL cholesterol levels Sniderman et al. [1] noted elevated LDL apo B. They termed this entity, characterized by 1) LDL cholesterol below 200 mg/dl, 2) Elevated LDL and apo B, 3) Low LDL cholesterol/apo B ratio,"hyperapobetalipoproteinemia". Apo B has been shown in prospective epidemiological studies to predict first Ischemic Heart Disease (IHD) event - Typical effort angina, Nonfatal myocardial infarction, and coronary death. Apo B levels have also been shown to predict risk of both recurrent ischemia and death among patients with Acute Coronary Syndrome.

Thus apo B has been proven to be a novel biochemical marker in primary prevention setting in predicting future cardiovascular events. Apo B may be regarded as a relevant tool in the assessment of IHD risk, because it may provide information that would not be obtained from the conventional lipid-lipoprotein profile. [17] This study was done to address the correlation between apo B levels and coronary angiographic findings in patients admitted for elective coronary angiography (CAG).

AIMS AND OBJECTIVES

AIM OF THE STUDY:

To determine the presence of an association between apo B and Coronary Artery Disease in Indian population.

OBJECTIVES OF THE STUDY:

1. To find out whether apo B is an independent risk factor for Coronary Artery Disease.

2. To see whether apo B is superior to routine lipid profile in differentiating patients with Coronary Artery Disease.

REVIEW OF LITERATURE

Over 90% of low density lipoprotein (LDL) particle is composed of apo B. It serves the function of solubalizing cholesterol within the LDL complex, which in turn increases the transport capacity of LDL for subsequent deposit on the arterial wall. Apo B is therefore a convenient marker for assessing the cholesterol depositing capacity of the blood.

Apolipoproteins (apoproteins) are a specialized group of proteins that associate with lipids and mediate several biochemical steps associated with plasma lipid metabolism. The apolipoproteins are designated by letters and Roman numerals, for example, apo A-I and apo C – II. The designations arose from two sources. First, it was once thought that apolipoproteins composed families, so those forming a family were given the same letter prefix. The scheme has remained even though its basis is no longer widely accepted. The numbers refer simply to the order in which the fractions that contain them emerge from a chromatographic column in their isolations.

The apolipoproteins encompass a wide range of molecular masses, from less than 6kDa for apo C – I to more than 500kDa for some B apolipoproteins and apo [a]. The plasma apolipoproteins can be divided in to three groups according to shared protein and gene structures. Apo B-100 and apo B-48 form the group 2 plasma apolipoproteins. The two apolipoproteins are grouped together because they are very large and are associated with the cholesteryl ester – rich lipoproteins and TGRLs (TriGlyceride Rich Lipoproteins).

Apo B-100 also contains several lipid-binding domains. They have been identified in a tryptic digestion, in heparin-binding domains, and in multiple sites of glycosylation. Apo B-100 contains 25 cysteines, some of which are located in a disulfide cluster within the first 500 amino acid residues of the primary structure. One of the cysteines form a disulfide link with apo [a]; this is the means by which apo [a] is attached to LDL. Apo B-48 is the amino terminal 48% of apo B-100. Although a single gene codes for both proteins, mRNA editing machinery in the intestine substitutes a stop codon for one that codes for an amino acid (Chen et al. 1987). Apo B-48 contains some of the glycosylation and heparin-binding sites as well as the disulfide cluster of apo B-100, but it does not contain the receptor-binding domain of apo B-100 that targets LDL to cell surface receptors.

Although all mature lipoproteins have a uniform surface monolayer of lipoproteins and phospholipids, there is considerable heterogeneity in apolipoprotein ratio depending on the metabolic state of the subject. Larger VLDL particles contain apo B-100, C apolipoproteins, and apo E. Smaller VLDL particles contain less C apolipoprotein. Virtually the entire protein component of Intermediate Density Lipoprotein (IDL) is apo B-100 and apo E. An LDL particle contains one molecule of apo B-100 as its sole protein. [18]

Apoproteins not only stabilize lipoprotein structure but also have other important regulatory functions in lipoprotein metabolism. Apolipoproteins B-100 and E are necessary for the binding of lipoproteins to cellular receptors, whereas apoproteins A-I and C-II are activators of enzymes important in lipoprotein metabolism.

Apolipoprotein measurements help to determine CAD risk such as apoprotein A-I (the major HDL protein) and apolipoprotein B (the major LDL protein). Measurement of apoprotein B may be clinically useful in certain situations. In a patient with CAD and an apparently normal cholesterol concentration, apo B measurement may be high. Apolipoprotein B is also helpful in the assessment of CAD risk in the hypertriglyceridaemic patient with a relatively normal cholesterol concentration. [19]

COMPARISON OF APO B TO OTHER RISK FACTORS:

Although the conventional approach to assessing the risk of CAD and the adequacy of therapy remains LDL cholesterol, there is compelling evidence that apo B is superior to LDL cholesterol for both of these purposes. The evidence can be grouped into 6 major categories, each of which is important, but each of which supports the others; and therefore, taken together, these 6 categories of evidence lead to straightforward and definitive conclusions.

The first major category consists of the studies that demonstrated that increased numbers of small, dense LDL are not only common in patients with CAD but, in fact, are much more common than increased LDL cholesterol.

The second major category of evidence includes the series of metabolic studies that have elucidated the metabolic basis for the increased numbers of small, dense LDL, which are so common in coronary patients. These studies have demonstrated that increased numbers of small, dense LDL are the result of increased secretion of very low-density lipoprotein (VLDL) by the liver. In addition, they have shown that shifts of cholesterol ester and triglyceride between VLDL and LDL and then hydrolysis of triglyceride within the LDL are responsible for the changes in composition.

The third category consists of the extensive series of in vitro and in vivo studies that have shown that small, dense, cholesterol-depleted LDL particles are more atherogenic than LDL particles of normal composition.

The fourth category of evidence includes the results of recent major epidemiological studies, which have demonstrated that apo B is a better index of the risk of coronary artery disease than LDL cholesterol. In the cross sectional study by Westerveld et al [20] where 289 women were followed up for 5 years, apo B was the best discriminant between CAD – and CAD +. Even among women in the lowest quartiles of Total cholesterol, LDL-cholesterol, and Triglyceride, who had angiographically proven CAD, apo B was significantly higher (See figure 1).

Apo B Levels In Lowest Quartiles Of Total Cholesterol, LDL – Cholesterol, Triglycerides And In The Highest Quartiles Of HDL – Cholesterol.

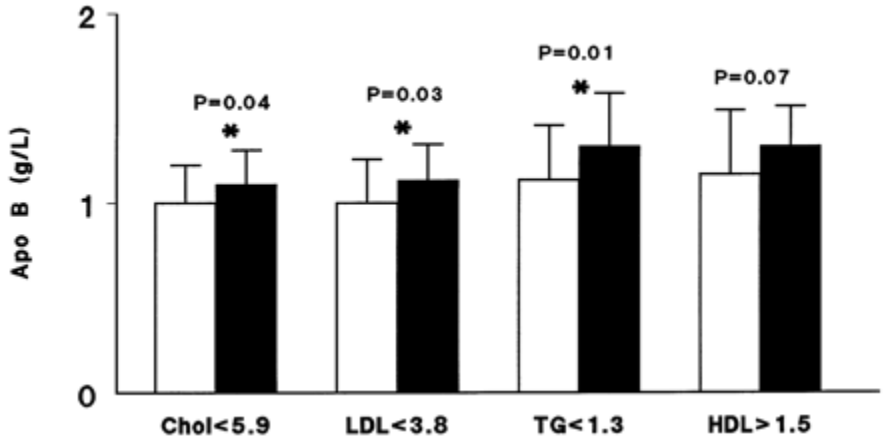


Figure 1: This shows plasma concentrations of Apo B (g/L) in the lowest quartiles of plasma cholesterol (<5.9 mmol / L), LDL – Cholesterol (< 3.8 mmol / L), and Triglycerides (<1.3 mmol / L) and in the highest quartiles of HDL – Cholesterol (> 1.5 mmol / L) in women with (CAD +, black bar) and without (CAD -, white bar) angiographically established CAD. p values were calculated with student's t test for independent samples.[20] (TG = Triglycerides)

As shown in figure 1 even among patients with lowest levels of LDL cholesterol (<3.8mmol / L), TGL (<1.3mmol / L) and highest levels of HDL cholesterol (> 1.5 mmol / L) apo B levels were significantly higher (p = 0.01) in women with CAD + (black bar) than in women without CAD (white bar).

The Quebec Cardiovascular Study [17] was a large prospective study to demonstrate strongly that apo B was superior to total or LDL cholesterol as an index of vascular risk. During the five-year follow-up of men in the Quebec Cardiovascular Study, Kaplan-Meier survival analysis across tertiles of apolipoprotein (apo) B and A-I levels expressed as the estimated probability for not having ischemic heart disease during the 5-year follow-up suggested that, men in the third tertile of the apo B distribution showed a

significant reduction in the probability of remaining free of IHD during follow-up compared with men in the first tertile, whereas the survival probabilities among tertiles of apo A-I were not statistically different (P>0.3), suggesting that apo A-I may not adequately predict IHD compared to apo B as shown in Figure 2.

Kaplan-Meier Survival Analysis Across Tertiles Of Apolipoprotein (Apo) B And A-I Levels

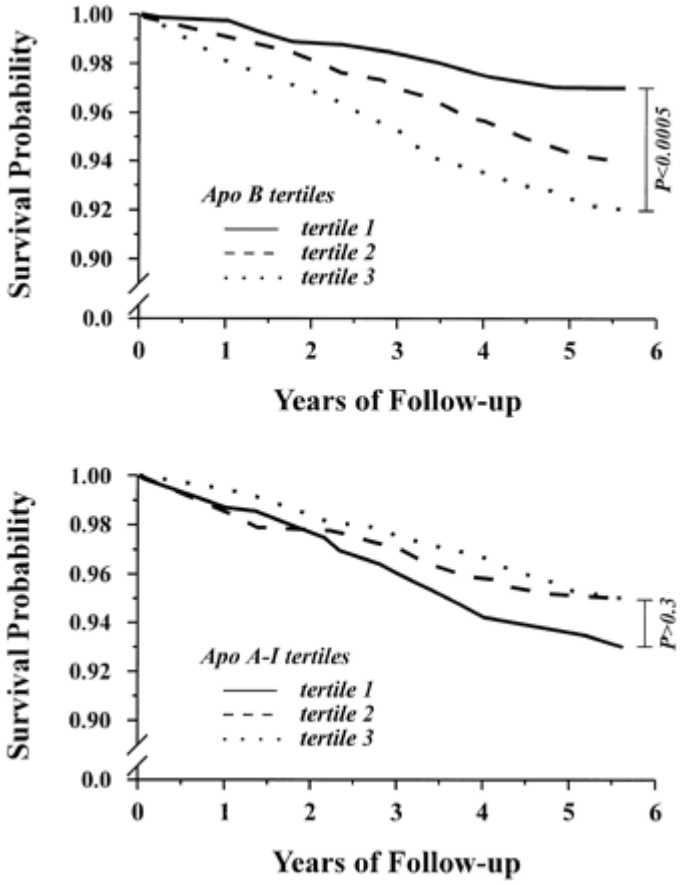


Figure 2: Kaplan-Meier survival analysis across tertiles of apolipoprotein (apo) B and A-I levels expressed as the estimated probability for not having ischemic heart disease during the 5-year follow-up of Men in the Quebec Cardiovascular Study. [17]

Figure 2 shows that, men in the third tertile of the apo B distribution had a significant reduction in the probability of remaining free of CAD during follow-up compared with men in the first tertile. Whereas such a difference could not be seen across all tertiles of apo A-I , suggesting that apo B is a better predictor of survival probability than apo A-I.

The Apoprotein-related Mortality Risk Study (AMORIS) study [21] is one of the largest studies and should be regarded as definitive on the issue of the merits of apo B versus LDL cholesterol, because its specific objective was to compare apo B and LDL cholesterol, and standardized, accurate methods were used to measure the lipoprotein lipids as well as apo B. In this study, 175,553 Swedes were followed for an average of 5.5 years. The clinical event was fatal myocardial infarction. Apo B was superior to LDL cholesterol in every direct comparison. Although apo B was superior to LDL at every level of cholesterol, the difference was particularly marked in those in the lower half of the distribution.

The fifth category of evidence supporting the argument that apo B is superior to LDL cholesterol is that, it is the calculation of LDL cholesterol, which has important methodological weaknesses, and not the measurement of apo B. The first weakness is that in order to obtain a plasma sample from which LDL cholesterol can be calculated, patients must be fasting, whereas they do not need to fast to measure apo B. Second, it is well known that LDL cholesterol cannot be calculated if plasma triglycerides are more than 400 mg/dL, and therefore, not infrequently, an estimate of LDL cannot be made.

However, it is not widely appreciated that calculated LDL cholesterol can differ importantly from true LDL cholesterol in patients with diabetes mellitus, in type 3 dyslipoproteinemia, nephrotic syndrome, and liver disease.

The sixth, and most recent, category of evidence showing the importance of apo B consists of the series of studies that demonstrate the level of apo B on statin treatment remains predictive of outcome, whereas that of LDL cholesterol generally does not.[22 - 25]. This is a major advantage for apo B. These data indicate that if therapy is guided by apo B rather than LDL cholesterol, statins have a much larger potential to reduce clinical events than is presently being achieved.

Both LDL and TGRLP (triglyceride-rich lipoproteins) contain apolipoprotein B-100 (apo B) as their major apolipoprotein. A growing view holds that most, if not all of apo B containing lipoproteins are atherogenic. Although different subspecies of apo B containing lipoproteins may vary in their atherogenic potential, a simplifying concept is that most of these subspecies carry similar atherogenicity. If so, then measurement of serum total apo B signifies the atherogenic potential of the whole lipoprotein fraction. Total apo B levels are clearly a strong predictor of CHD risk.

Total apo B levels correlate relatively strongly with non-HDL cholesterol levels. [23,24] The correlation is particularly strong in the absence of elevated serum TGL, but weakens somewhat as TGL levels rise. Still, non-HDL cholesterol includes all of the cholesterol in apo B-containing lipoproteins. Because there is one apo B molecule per lipoprotein

particle, total apo B concentrations are a measure of total particle number in LDL+TGRLP, whereas non-HDL cholesterol provides the cholesterol content of these same lipoproteins. Whether total apo B or non-HDL cholesterol is a better predictor of CHD risk has not been determined through robust prospective studies.

Non-HDL Cholesterol (or Total Apo B): Replacement for LDL Cholesterol?

A few investigators [25] propose that non-HDL cholesterol or its correlate, total apo B, should replace LDL cholesterol in clinical cholesterol guidelines. See Table 1.

Table 1: Treatment Goals for LDL-C, Non-HDL-C, and Total Apo B[26]

	Therapeutic Goal, mg/dL		
Risk Status	Primary Target: LDL Cholesterol	Secondary Target: Non-HDL Cholesterol*	Secondary Target: Total Apolipoprotein B†
CHD‡ and CHD risk equivalents‖	<100	<130	<90
Multiple (2+) risk factors$	<130	<160	<110
Zero to 1 risk factor	<160	<190	<130

*Non-HDL cholesterol becomes a secondary target of therapy when serum triglycerides range from 200 to 500 mg/dL.

†Apolipoprotein B is an alternate secondary target of therapy when serum triglycerides range from 200 to 500 mg/dL.

‡Includes a history of myocardial infarction, unstable angina, stable angina, coronary artery procedures, and clinical evidence of myocardial ischemia.

‖Includes clinical forms of non-coronary atherosclerotic vascular disease (peripheral arterial disease, abdominal aortic aneurysm, clinical carotid artery disease), diabetes, and multiple (2 or more) risk factors with 10-year risk for major coronary events (myocardial infarction+coronary death) ≥20%.

$Multiple (2 or more) risk factors with 10-year risk for major coronary events ≤20%. ATP III modifies intensity of LDL-lowering therapy required to achieve the goals of therapy according to 10-year risk for CHD.

The table 1 summarizes ATP III's therapeutic goals for LDL cholesterol and non-HDL cholesterol. Also shown are corresponding goals for total apo B, which are derived from the known relationship between total apo B and non-HDL cholesterol.

Thus total apo B represents an alternative secondary target of therapy. [26] There is growing interest in the possibility that additional risk reduction may be obtained by incremental lipid-lowering therapy after ATP III's goals for LDL cholesterol have been met. If so, the next logical therapeutic target is non-HDL cholesterol or total apo B.

In the study by Tobias Pischon at al [27] both apo B and non–HDL-C have been proposed as markers to reflect the risk conferred by proatherogenic triglyceride rich VLDL in addition to LDL-C. [28, 29] Apo B was associated with increased risk of CAD even after adjustment for LDL-C or non–HDL-C, despite the high degree of correlation between these variables. Furthermore, triglyceride levels provided additional information on CHD risk beyond LDL-C and non–HDL-C but not apo B levels.

In the Nurses' Health Study, [30] apo B was more strongly related to CAD than was LDL-C. Among persons with low non–HDL-C (<139.6 mg/dL) a mid-range level compared with a low-range level of apo B increased CAD risk by 55%. This clinical phenotype, normocholesterolemic hyper-apo B, has been previously described and hypothesized to have a high risk of CAD.[31] In those with mid-range non–HDL-C (139.6 to 171.3 mg/dL), a high compared with a low apo B level increased risk by & 2.4-fold. In contrast, when apo B was used for the primary risk classification, non–HDL-C levels did not affect risk.

APO B AND CARDIOVASCULAR RISK: EPIDEMIOLOGIC AND CLINICAL EVIDENCE:

The long-term 13-year follow-up data from the Quebec Cardiovascular Study [17] which evaluated the association between apo B levels and CAD risk indicated that high plasma apo B and LDL cholesterol concentrations were equally and independently associated with an increased long-term relative risk of CAD. It was found that high apo B and LDL cholesterol levels are associated with an approximately 2-fold increase in the long-term relative risk of CAD after adjustment for nonlipid and lipid variables.

Several studies have indicated that increased apo B levels may be associated with a greater risk of CAD compared with high LDL cholesterol concentrations. [32,33]

In the study by Westerweld et al [20] it was shown that in women undergoing their first Coronary angiography, those with lower levels of apo B <0.7 g/L had no CAD whereas when the levels of apo B increased there were more women with CAD. When levels of apo B were more than 2 g/L there were no women without CAD as shown in the following frequency distribution curve (See Figure 3).

Frequency Distributions Of Plasma Apo B Concentrations In Women With And Without CAD.

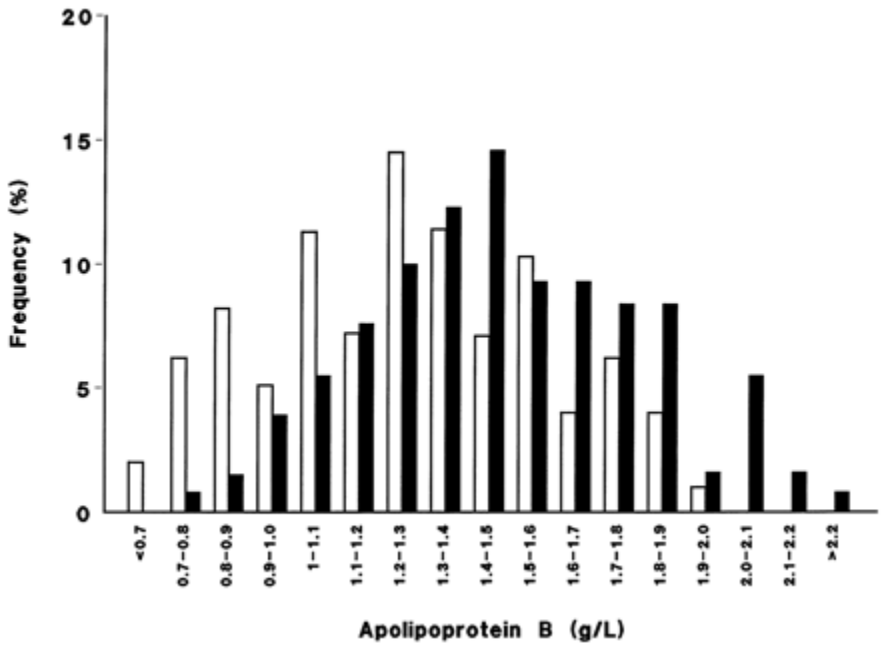


Figure 3: Frequency distributions of plasma apo B concentrations in women with (CAD+, black bar) and without (CAD-, white bar) angiographically established CAD. [20]

In the above figure 3, it is seen that as the levels of apo B increased the frequency of patients with CAD increase. In the group with apo B < 1 g / L there are more CAD – patients (white bar) and in the group with apo B > 1 g / L there are more CAD + patients (black bar).

Results from Walldius et al[21] showed that apo B had a higher sensitivity and specificity than did LDL cholesterol in predicting future coronary death in men and women in the Apolipoprotein-related Mortality Risk Study (AMORIS) [21]. (See figure 4). The specific aim of the AMORIS study was to compare apo B and LDL cholesterol. In this study apo B was superior to LDL at every level of LDL cholesterol, specifically in those with lower LDL levels .

Apo B Versus LDL – Cholesterol In Predicting The Mortality Risk Ratio

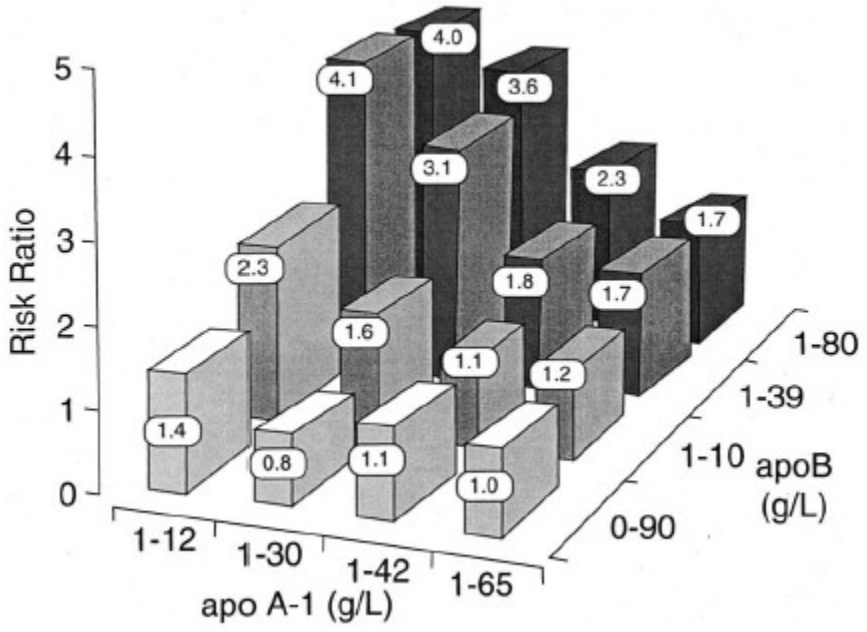


Figure 4: Results in men <70 years from the Apoprotein-Related Mortality Risk Study (AMORIS). The risk ratios were calculated after adjustment for cholesterol and triglycerides and age. Apo = apolipoprotein. [21]

Figure 4 presents the risk attributable to apo B and apo A1 in men <70 years after the effects of cholesterol and triglyceride have been taken into account. The figure depicts the additional substantial predictive power provided by the apoproteins above that provided by the lipids. Note that risk increases both as apo B increases and apo A1 decreases.

Large-scale trials with clinical end points have established that statin therapy decreases the risk of CAD events in primary and secondary prevention settings. However, analysis of data of the statin group in the Long-term Intervention with Pravastatin in the Ischemic Disease (LIPID) trial showed that levels of apo B after therapy were a better predictor of CAD risk compared with LDL cholesterol.

The persisting increased levels of atherogenic particles that were not captured by measurement of LDL cholesterol alone suggests that even what has been considered aggressive lipid-lowering therapy may not be aggressive enough to optimize risk decrease in some patients. [34] This suggests that therapy that focuses not only on LDL cholesterol levels but also on apo B concentrations may be useful to more adequately decrease the risk of CAD in the general population.

THE POPULATION DISTRIBUTION OF APO B

Recently two American studies have given the distribution of apo B in general population. Contois and co-workers [35] presented reference intervals for apo B derived from the population-based Framingham Offspring Study. The mean (±SD) apo B

concentration was 1.03 ± 0.24 g/L in 1880 men, significantly higher than the mean for 1944 women (0.96 ± 0.26 g/L). Subjects with concentration of apo B greater than 1.20g/L were significantly more likely to have CHD (Coronary Heart Disease) than subjects with apo B concentrations less than 1.00 g/L.

Bachorik et al.[36] measured the concentration of apo B in Phase 1 (1988 –1991) of the Third National Health and Nutrition Examination Survey (NHANES III) and found that among US civilian population who were 4 years of age or older, apo B concentrations were low in childhood (~0.80 g/L) and increasing to ~1.2 g/L in adults.

A large study done in Sweden, with a sample size of 1,47,576 patients the mean value (±SD) for apo B was 1.31 ± 0.35 g/L in all males and 1.22 ± 0.36 g/L in all females. The mean value of apo B increased up to 60 years of age in males and up to 70 years of age in females. (See figure 5).

Median Apo B Values In Different Age Groups In Males And Females

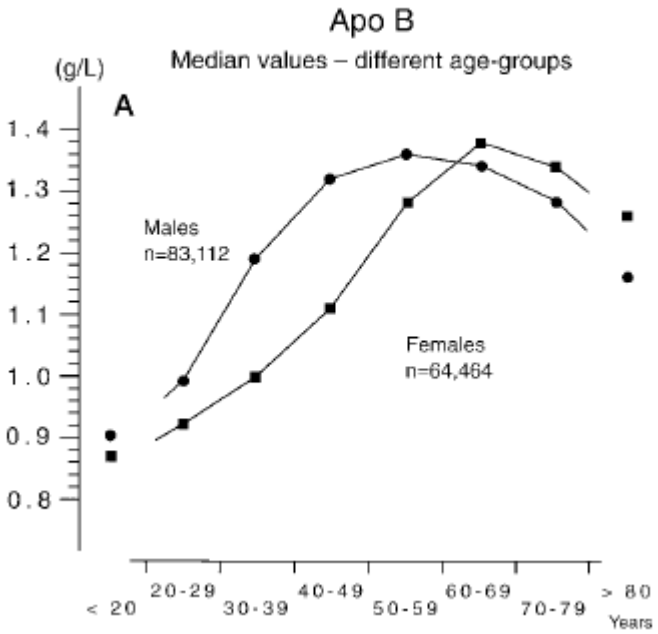


Figure 5: This figure shows the changing apo B levels with increasing age in males and females. [37]

As shown in figure 5 in the Swedish study median apo B concentrations in adult males increased from 20 – 59 years of age and tended to decrease after the age of 60 years whereas among females apo B increased continuously from 20 – 69 years and then decreased after age 70

THERAPEUTIC INTERVENTIONS

A number of studies have shown the benefit of reducing apo B in patients with CAD. These trials included life style modifications and pharmacological interventions mainly statins.

BEHAVIOURAL INTERVENTION:

Weight Loss:

Visceral obesity is frequently associated with high plasma triglycerides and low plasma high density lipoprotein-cholesterol (HDL-C), and with high plasma concentrations of apolipoprotein B (apo B)-containing lipoproteins. Atherogenic dyslipidemia in these patients may be caused by a combination of overproduction of very low density lipoprotein (VLDL) apo B-100, decreased catabolism of apo B-containing particles, and increased catabolism of HDL-apoA-I particles. These abnormalities may be consequent on a global metabolic effect of insulin resistance. Weight reduction, increased physical activity, and moderate alcohol intake are first-line therapies to improve lipid abnormalities in visceral obesity. These lifestyle changes can effectively reduce plasma triglycerides and low density lipoprotein-cholesterol (LDL-C), and raise HDL-C. Kinetic studies show that in visceral obesity, weight loss reduces VLDL-apo B secretion and reciprocally upregulates LDL-apo B catabolism, probably owing to reduced visceral fat

mass, enhanced insulin sensitivity and decreased hepatic lipogenesis.

Physical Activity

Regular physical activity may favourably moderate the lipid levels. It has been found that total cholesterol, LDL-cholesterol and apo B concentrations were lower in lean exercisers than in lean sedentary men, suggesting that exercise influences these risk factors.

Indeed, time spent in vigorous activity has been noted to significantly predict total cholesterol and LDL-cholesterol. Leanness has been observed to be associated with favourable levels of HDL-C and triglycerides, while exercise was associated with lower levels of total cholesterol, LDL-cholesterol and apo B.

Smoking cessation:

Cigarette smoking is an important risk factor for CAD. The apolipoprotein A-I has been significantly lower and apolipoprotein B significantly higher in smokers than non-smokers. It has also been noted that those who smoked > 20 cigarettes/day had higher apolipoprotein B levels than those who smoked < 10 cigarettes/day and non-smokers.

The effect of cessation of smoking in reduction of apo B is less clear and larger clinical trials are warranted in this aspect.

Dietary Factors:

Studies on dietary factors known or hypothesized to confer cardio protection or harm and inflammatory markers have begun to provide information about their relations, but a clear picture has not emerged. This section provides a selective review of recent dietary findings.

ω3 Fatty Acids:

Observational and intervention studies suggest that fish intake reduces CAD incidence and mortality. Such cardio protection might be explained by an effect of long chain ω3 polyunsaturated fatty acids found in fish (and certain plant oils).

In the study by Laura Calabresi et al [38] Fourteen Familial Combined Hyper Lipidemia patients received 4 capsules daily of ω-3 polyunsaturated fatty acid [ω3 FA] concentrate providing 1.88 g of eicosapentaenoic acid [EPA] and 1.48 g of docosahexaenoic acid [DHA] per day or placebo for 8 weeks in a randomized, double blind, crossover study. Plasma triglycerides were 44% lower, and LDL cholesterol and apolipoprotein (apo) B were 25% and 7% higher after ω-3 polyunsaturated fatty acid capsules than placebo.

In another similar study [39] where 14 patients received Omacor, a drug containing the n-3 fatty acids eicosapentaenoic and docosahexaenoic acid (EPA and DHA), or placebo for 8 weeks in a randomized, double blind, crossover study. Omacor significantly lowered plasma triglycerides and VLDL-cholesterol levels, by 27 and 18%, respectively. Total cholesterol did not change but LDL-cholesterol and apolipoprotein B (apo B) concentrations increased by 21 and 6%.

Results from several small scaled randomized trials of omega 3 fatty acids supplementation are inconsistent. Larger scale trials in higher risk populations are warrented.

Low fat low cholesterol diet:

Excess body weight can result in changes in plasma lipids and lipoproteins that increase the risk of atherosclerotic cardiovascular disease (CVD). Dietary carbohydrates, especially simple sugars, can also promote atherogenic dyslipidemia, in large part because of effects on the metabolism of plasma triacylglycerol-rich lipoproteins. Reductions in dietary carbohydrate intake in diets are achieved both by reductions in total calorie intake and by substitution of protein, fat, or both. In a study by Ronald M Krauss et al [40] the effects of moderate reductions in carbohydrate intake on atherogenic dyslipidemia in overweight and mildly obese men was tested. In addition, the extent to which changes in lipoprotein with reductions in carbohydrate intake are influenced by

variations in saturated fat content was also analyzed.

All subjects consumed the basal diet (54% of energy as carbohydrate) for 1 wk, after which they were randomly assigned to the basal diet (54% carbohydrate, low saturated fat diet) or 1 of the 3 low-carbohydrate diets, which included 39% carbohydrate, low saturated fat diet or 26% carbohydrate, low saturated fat diet or 26% carbohydrate, high saturated fat diet.

The 26%-carbohydrate, low-saturated-fat diet resulted in reductions from baseline in total cholesterol, triacylglycerol, apo B levels. A lower carbohydrate intake was associated with reduced plasma apo B concentrations and a reduced ratio of total cholesterol to HDL. With weight loss, there were reductions in major lipid and lipoprotein indicators of CVD risk (LDL cholesterol, triacylglycerol, apo B, total: HDL cholesterol, and small, dense LDL).

Vitamins:

Supplementation with vitamin E has beneficial effects on oxidative stress and (surrogate) markers of atherosclerotic disease but the results on clinical end-points in general have been disappointing. A recent meta-analysis on this subject concluded that there is no indication for routine use of antioxidants in order to improve cardiovascular outcome. [41] In a prospective, double blind randomised placebo-controlled trial of effects of treatment with atorvastatin and α-tocopherol in 44 clinically stable, non-diabetic patients on dialysis therapy on apo B and oxidative stress was analyzed. [42] Assessment of lipid profile and oxidative stress was performed at the start of the study and after 12 weeks of treatment.

Treatment with atorvastatin reduced total cholesterol, triglycerides (TG), low-density lipoprotein (LDL) cholesterol, apolipoprotein B (apo B) and levels of oxidized LDL (oxLDL) with 30-43%. It had no influence on LDL oxidisability. Additional supplementation with alpha-tocopherol had no effect on lipid profile and oxidized LDL levels and apo B, but decreased in vitro LDL oxidisability. Supplementation of alpha-tocopherol to atorvastatin had beneficial effects on in vitro LDL oxidisability and might therefore be of additional value. Thus the effects of vitamins on apo B levels have not been well established and require larger studies.

Alcohol use:

The concentrations of HDL cholesterol, total plasma triglycerides, apo B were significantly higher in the alcohol abusers than the control subjects. [43] J. Michael Gaziano et al [44] examined the interrelation among alcohol consumption, plasma lipoprotein levels, and the risk of myocardial infarction in 340 patients who had had myocardial infarctions and an equal number of age- and sex-matched controls. He observed a significant inverse association between alcohol consumption and the risk of myocardial infarction (P for trend, <0.001 after control for known coronary risk factors). Apolipoproteins A-I and A-II were also positively associated with alcohol consumption (P for trend, <0.001 for both A-I and A-II), whereas there was no such relation for apolipoproteins B and E.

The effect of alcohol consumption on the concentrations of apolipoproteins was evaluated in a study by Ikunosuke Sakurabayashi et al. [45] Even in this study apo B and apo B/apo A-I were significantly higher (p<0.001) in women who consumed alcohol (drinking subjects) than in women who did not consume alcohol (nondrinking subjects). However, in the total subjects and in the men, no significant difference in apo B was detected between drinking and nondrinking subjects. Thus it appears that alcohol abuse

increases apolipoprotein concentrations, but moderate drinking may not influence apolipoprotein concentrations.

PHARMOCOLOGIC INTERVENTIONS:

Several pharmacological agents with demonstrated cardio protective ability reduce apo B levels.

LIPID MODULATING AGENTS:

Lipid modulating medications reported to affect apo B levels include 3 – hydroxy – 3 – methylglutaryl coenzyme A reductase inhibitors (statins), fibrates and niacin. Of these, the findings for statins are by far the most robust.

Statins:

In the CARDS study [46] 2838 patients were randomized to atorvastatin or placebo for evaluation of effectiveness and safety of atorvastatin 10 mg daily versus placebo in the primary prevention of cardiovascular disease (CVD) in patients with type 2 diabetes, LDL cholesterol levels ≤4.14 mmol/L and triglycerides ≤6.78 mmol/L. The average length of the trial was 3.9 years. The apo B levels were decreased by 23% in the atorvastatin arm compared to placebo, resulting in 37% decrease in CVD events, which was evident after 18 months.

In the Atorvastatin Comparative Cholesterol Efficacy and Safety Study (ACCESS), [47] whereas LDL cholesterol decreased from around the 90th percentile for the population to below the 25th percentile, apo B that was also around the 90th percentile was decreased only to around the 50th percentile. The reason why LDL is reduced more than apo B by statins is that because there is a single molecule of apo B in every LDL particle, serum apo B concentrations reflect the LDL particle concentration. So clearly the LDL particle

concentration does not decline as much on statin therapy as is suggested by the degree of reduction in LDL cholesterol.

There are two mechanisms of action of statins on apo B: one diminishing apo B by the removal of more buoyant LDL and the other shifting the distribution of apo B from smaller denser particles to larger more buoyant LDL. The response of apo B to statins would thus appear to reflect both the decrease in buoyant LDL and SD-LDL (Small Dense – LDL). On the other hand, the decrease in LDL cholesterol reflects more closely the effect of statins on more buoyant LDL. Because it is believed that the SD-LDL constitutes a greater risk for atherosclerosis, monitoring statin therapy with an apo B target as opposed to an LDL cholesterol target may be a more effective means of optimizing statin therapy. The association of LDL cholesterol with atherosclerosis appears to be the result of chemical modification of the apo B component of the LDL particle, allowing it to become a ligand for the scavenger receptors of arterial wall monocyte-macrophages leading to its rapid uptake and foam cell formation. It is perhaps therefore not surprising that apo B is a better indicator of CAD risk than LDL cholesterol and it is increasingly appealing to monitor treatment by measuring apo B, the LDL component most directly involved in atherogenesis.

In a 16-week multinational trial, [48] 1993 high-risk patients were randomized to rosuvastatin 20 mg, atorvastatin 10 mg, atorvastatin 20 mg, simvastatin 20 mg, or simvastatin 40 mg for 8 weeks. Patients either remained on starting treatment or switched to lower or milligram-equivalent doses of Rosuvastatin for 8 more weeks. Achieving an apo B of <90 mg/dL in patients with elevated triglycerides was much more difficult than achieving both LDL-C <100 mg/dL and non–HDL-C <130 mg/dL. Conversely, achieving an apo B of <90 mg/dL guaranteed achieving the dual LDL-C and non–HDL-C targets.

Switching from statins that are less effective in reducing LDL-C, particularly when they are approaching their maximal dose, to those that are more effective in their lower dose range constitutes an important treatment option for optimizing achievement of ATP III goals in high-risk patients. This trial showed that switching from the most commonly used doses of atorvastatin or simvastatin to rosuvastatin at the

recommended starting dose (10 mg) or optional starting dose (20 mg) led to greater achievement of the proposed apo B target in hypertriglyceridemic patients. The magnitude of the benefit achieved with switching to rosuvastatin in these high-risk patients is consistent with treatment differences observed in comparative trials of rosuvastatin and other statins at commonly used doses. [49,50] Thus Rosuvastatin therapies produces greater reductions in apo B levels compared to atorvastatin or simvastatin.

Fibrates:

Small trials of varying designs have shown that fibrates may also decrease plasma concentrations of apo B in hyperlipidemic patients. The serum apo B concentration comparatively undergoes only minimal change by fibrates. [51] On the other hand, fibrate treatment is associated with substantially decreased Small Dense (SD)-LDL. [52,53] The relative lack of change in total serum apo B is thus due to a shift from SD-LDL to larger LDL. The decrease in SD-LDL on fibrates is due to the decrease in CETP (cholesteryl ester transfer protein) activity, which they induce probably as a consequence of a decreased pool of circulating VLDL. [54] The lack of change in serum apo B levels on fibrates clearly does not reflect the decrease in SD-LDL and the serum triglyceride response is likely to give a better indication of the probable change in SD-LDL. It is possible that apo AI, because of its inverse relationship with triglycerides and thus with SD-LDL, may, when expressed as a ratio with apo B, integrate the information about atherosclerotic risk in patients on fibrates, but direct evidence for this is largely lacking at present.

Niacin:

Data from small trials also suggest that niacin may reduce apo B levels. In a double-blind study [55] of 146 men who had apolipoprotein B levels greater than or equal to 125 mg per deciliter, documented coronary artery disease, and a family history of vascular disease, with a 2 1/2-year follow up, patients were randomly assigned to one of three treatments: lovastatin (20 mg twice a day) and colestipol (10 g three times a day); niacin (1 g four times a day) and colestipol (10 g three times a day); or conventional therapy with placebo

(or colestipol if the low-density lipoprotein [LDL] cholesterol level was elevated). The levels of LDL and high-density lipoprotein (HDL) cholesterol changed only slightly in the conventional-therapy group, but more substantially among patients treated with lovastatin and colestipol or niacin and colestipol. In the conventional-therapy group, 46 percent of the patients had definite lesion progression (and no regression) in at least one of nine proximal coronary segments; regression was the only change in 11 percent. By comparison, progression (as the only change) was less frequent among patients who received lovastatin and colestipol (21 percent) and those who received niacin and colestipol (25 percent), and regression was more frequent.

Clinical events (death, myocardial infarction, or revascularization for worsening symptoms) occurred in 10 of 52 patients assigned to conventional therapy, as compared with 3 of 46 assigned to receive lovastatin and colestipol and 2 of 48 assigned to receive niacin and colestipol.

Thus to conclude niacin given with colestipol to patients with apolipoprotein B levels greater than or equal to 125 mg per deciliter & documented coronary artery disease, similar to lovastatin with colestipol, in men with coronary artery disease who were at high risk for cardiovascular events, reduced the frequency of progression of coronary lesions, increased the frequency of regression, and reduced the incidence of cardiovascular events.

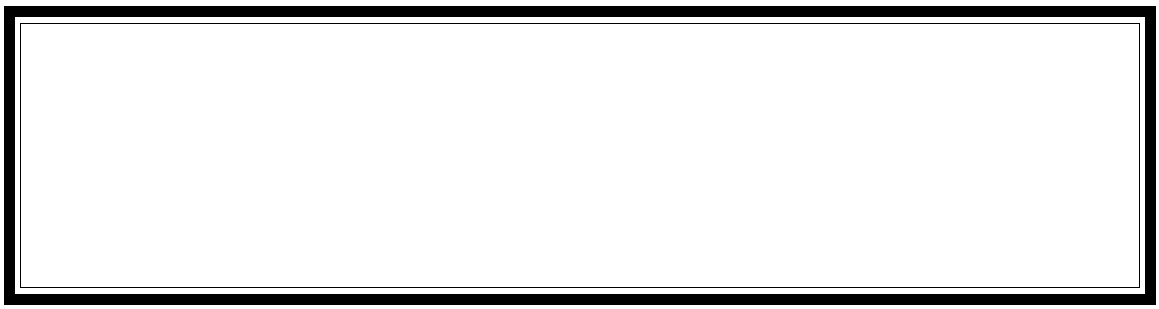
APO B AND CARDIOVASCULAR PROGNOSIS IN SECONDARY PREVENTION SETTINGS:

Apo B is predictive of recurrent vascular events among patients with Acute Coronary Syndrome and among patients with documented CAD.

Acute coronary syndromes:

James P. Corsetti et al [56] analysed 272 post infarction patients without history of diabetes from the Thrombogenic Factors and Recurrent Coronary Events (THROMBO) study. A proportional hazards model applied to dichotomized laboratory parameters demonstrated only apo B as a significant predictor of risk for recurrent coronary events in patients with metabolic syndrome with hazard ratio, 1.97 (95% CI; 1.08, 3.60; $P < 0.05$). He concluded that apo B is significantly associated with risk for recurrent coronary events in postinfarction patients with metabolic syndrome; and further studies would be needed to recommend the routine determination of apo B in these patient groups.

Arthur J. Moss et al [57] during a 4-year period (1994–1998), enrolled 1045 patients after recovery from an index myocardial infarction. Average follow-up period was 26 months. High concentrations of apo B were associated with recurrent coronary events in the absence of any identified risk from high concentrations of total cholesterol, LDL cholesterol, lipoprotein (a), or triglycerides. Small dense lipoprotein particles are thought to contribute to the lipid core of atherosclerotic plaques [58] and may be a factor driving the atherosclerotic plaque to instability, with recurrent coronary events related to consequent plaque deterioration.[59]



METHODOLOGY

The study was conducted in the Department of Cardiology at the Christian Medical College, Vellore.

Inclusion Criteria:

The study population consisted of patients who were admitted to Cardiology wards for Coronary Angiography. The indications for angiography were suspicion of Coronary Artery Disease (CAD) or preoperative screening for Coronary Artery Disease in subjects with valvular disease.

Exclusion Criteria:

Patients undergoing angiography during hospitalization for acute myocardial infarction or acute coronary syndrome were excluded to avoid the influence of these stress situations on plasma lipids.

Clinical and Lifestyle Characteristics:

All patients were screened by a thorough history and physical examination. Baseline characteristics such as hypertension, diabetes and smoking were taken from all the patients in a standard proforma.

Lipids and Apolipoproteins:

After an overnight fasting, blood samples were taken for lipids and apolipoproteins. The fasting lipid profile was estimated with Hitachi 912 analyzer. The LDL was calculated using the formula of Friedewald (Total Cholesterol – [HDL - cholesterol]-[0.45xTriglyceride]). Apo B was measured using immunoturbidimetric assay using the Orion Diagnostica. The later company is one of the manufacturers that have participated in the WHO – Capital IFCC standardization programme.

Coronary Angiography:

Coronary angiographies were performed according to the standard Judkins techniques. A patient was said to have CAD if there was an angiographic lesion more than 50% lesion. A patient was considered as control, if angiogram was normal or treadmill stress testing was negative.

Statistics:

- Baseline characteristics are presented as percentage of mean (Standard Deviation) and frequency for discrete variables.

- The Pearson correlation coefficient was used to analyze the presence of any correlation between apo B level, baseline characteristics and CAD.

- Presence of an association between various risk factors & apo B with CAD was analyzed using chi – square test for univariate analysis and binomial logistic regression for multivariate analysis.

- For all analysis significance was considered at a p value of <0.05.

- Student's t tests for independent samples were used to analyse differences in apo B concentrations between CAD – and CAD + groups.

- Both univariate and multivariate analysis were performed using SPSS for Windows, version 11 (SPSS, Inc., Chicago, Illinios) with the assistance of an experienced biostatistician.

RESULTS

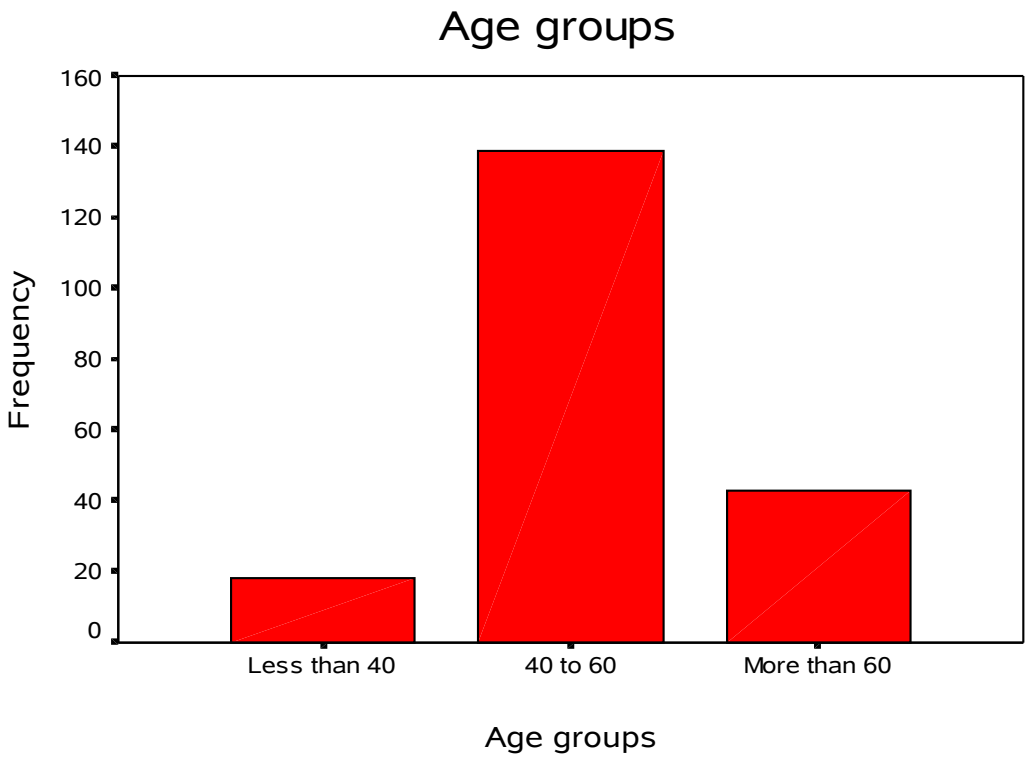
Subject Population and demographics:

A total of 200 patients were enrolled who had base line demographic information and a baseline apo B level.

1. Baseline Characteristics:

a) Age: The mean age was 53.3 years ranging from 24 years to 78 years.

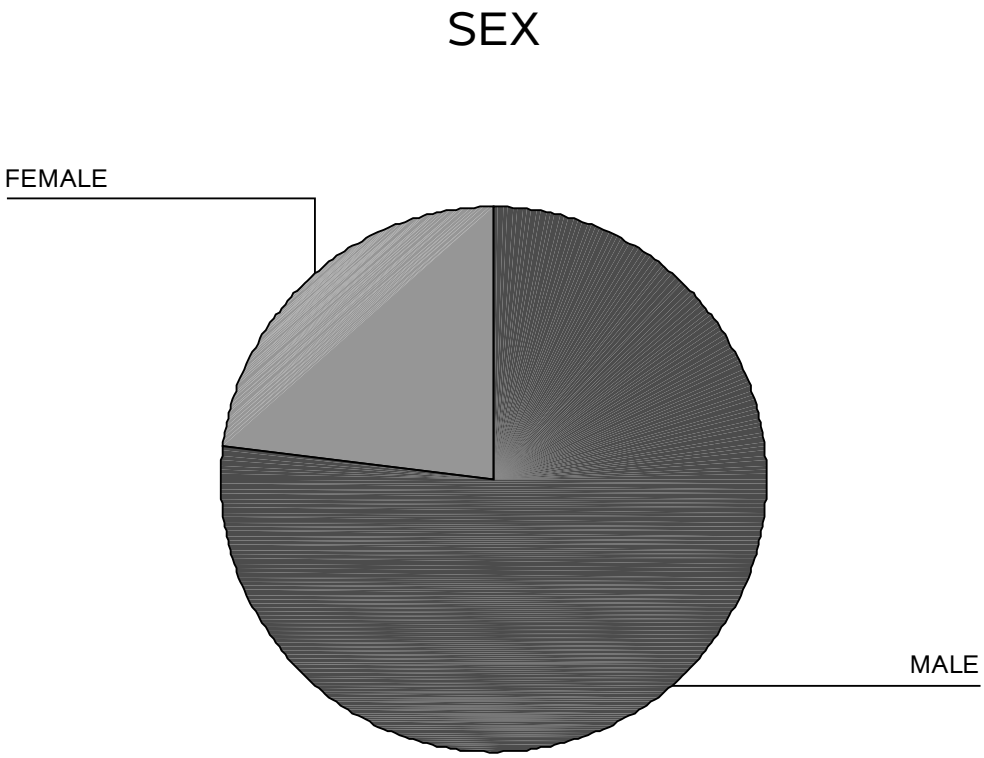
The following graph shows the distribution of cases.



Most patients were more than 40 years reflecting the greater prevalence of risk factors in

this age group.

b) Sex: There were 154 male patients (77%) and 46 female patients (23%).



2. Risk Factors:

The following table shows the percentage of patients who had various risk factors.

RISK FACTORS	FREQUENCY	PERCENTAGE
Diabetes Mellitus	82	41%
Hypertension	107	53.5%
H/O Smoking	73	36.5%
Past h/o MI/ACS	27	13.5%
Family h/o IHD	24	12%

MI – Myocardial Infarction

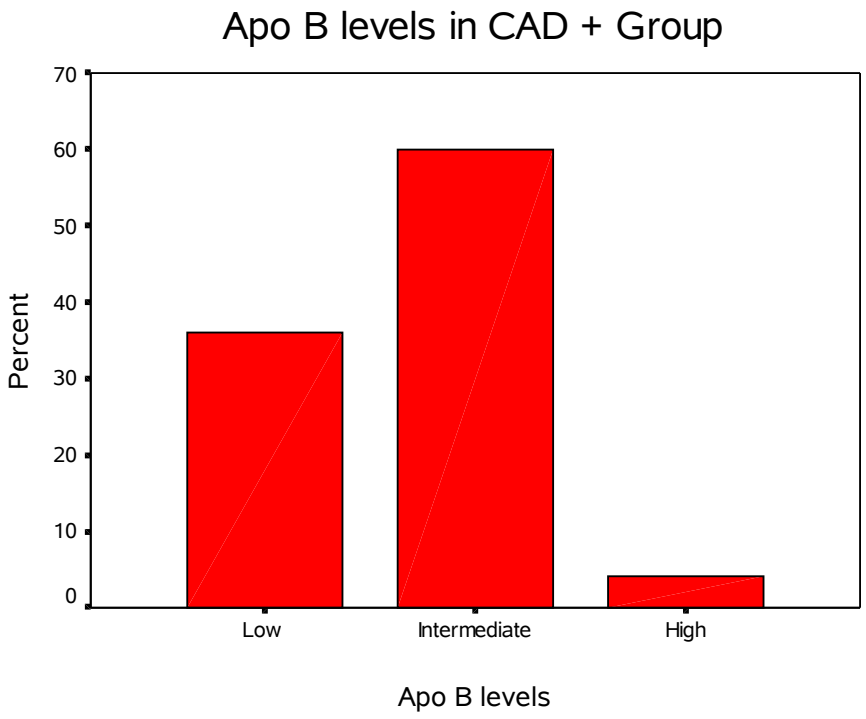
IHD – Ischemic Heart Disease

The percentage of patients having Low HDL, High TGL and High LDL were 99 (49.5%), 87 (43.5%) and 23 (11.5%) respectively.

3. Apo B and CAD:

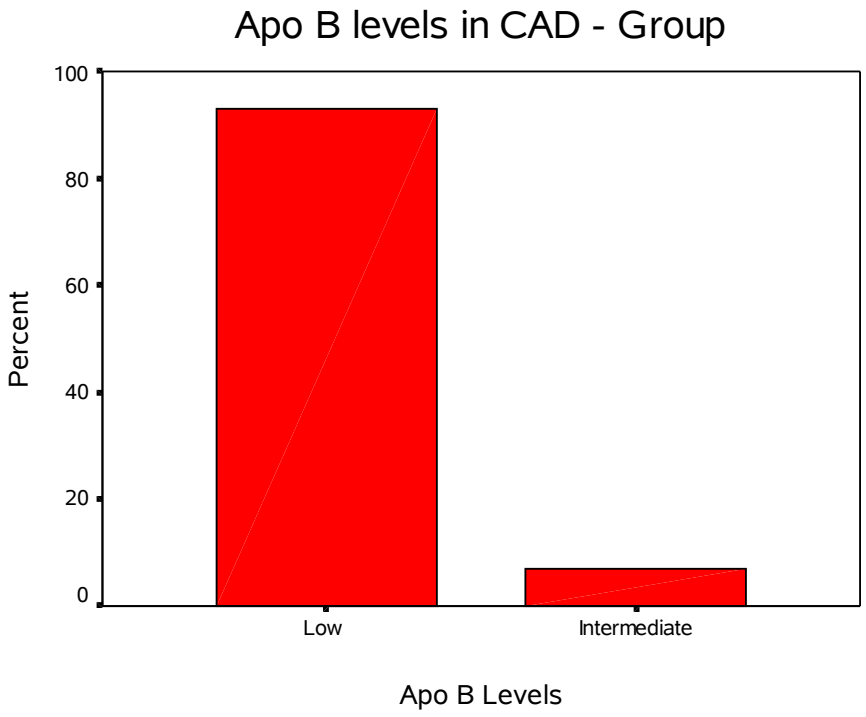
a) The median apo B level in the patients studied was 0.89 g/l with range of 0.11 to 3.00g/l.

b) The following chart shows the distribution of patients with low (< 1 g/l), intermediate (1 to 2 g/l) and high (>2 g/l) apo B levels among patients with Coronary Artery Disease (CAD).

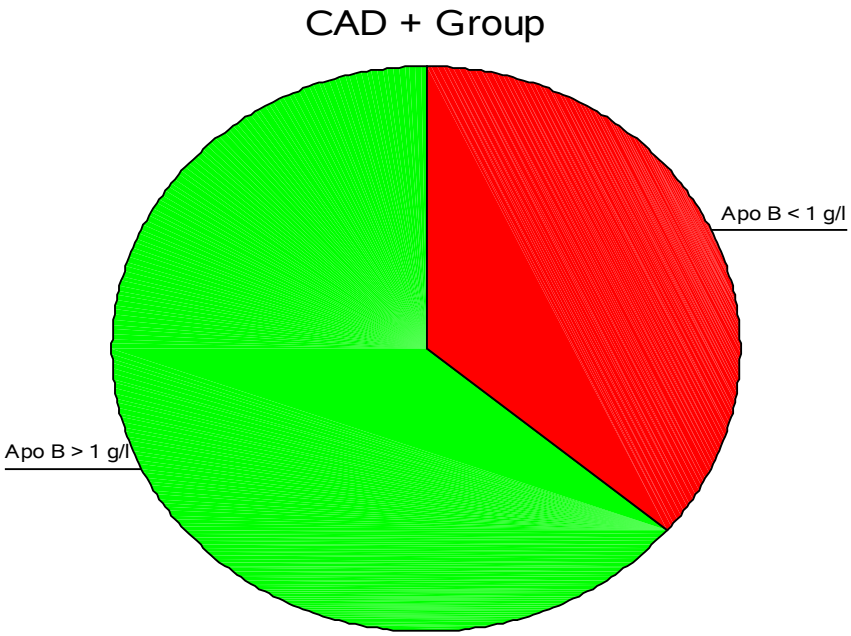


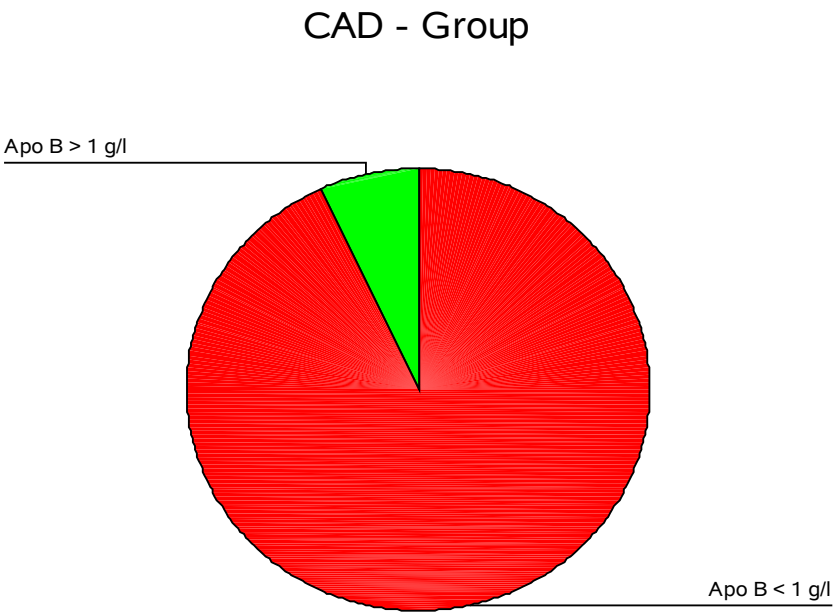
c) The following chart shows the distribution of patients with low (< 1 g/l), intermediate (1 to 2 g/l) and high (>2 g/l) apo B levels among patients without Coronary Artery Disease (CAD).

None of the patients in the CAD – group had an apo B value more than 2 g/l.



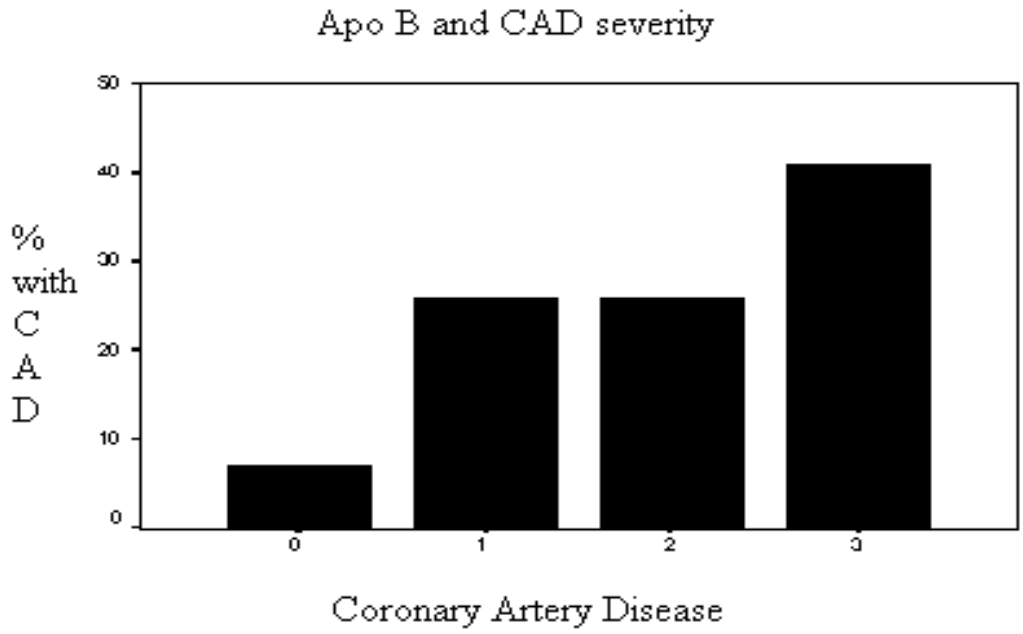
d) 64% of patients in the CAD group had apo B levels >1 g/l.whereas only 7% of patients in the group without CAD had apo B levels >1 g/l.





4. CAG findings:

The following chart shows the distribution of patients with CAD according to severity.



0 = Minor CAD

1 = Single Vessel Disease (SVD)

2 = Double Vessel Disease (DVD)

3 = Triple Vessel Disease (TVD)

Significant involvement of coronary artery was taken as more than 50% stenosis, < 50 % stenosis was included in the minor CAD group. 7% had Minor CAD 26% had SVD, 26% had DVD and 41% had TVD.

5. CAD and Traditional Risk Factors:

Association between traditional risk factors like age, gender, smoking, hypertension, diabetes mellitus, dyslipidemia, family history of CAD and past history of MI was analysed using the chi – square test that is summarized below.

Risk Factors	Pearson Square Value	p value
Age	6.64	<0.025
Sex	13.67	<0.001
Smoking	4.85	<0.05
Hypertension	2.43	<0.20
Diabetes Mellitus	2.07	<0.20
Family h/o CAD	1.71	<0.20
Total Cholesterol (TC) >200	7.79	<0.01
LDL > 130	5.94	<0.025
TGL > 150	5.87	<0.025
HDL < 40	3.38	<0.10
Apo B > 0.995	72.16	<0.001

Among the association between risk factors and CAD it was found that apo B was the most significant. Age, sex, smoking were also significantly associated with CAD.

The association of Apo B was more significant than LDL, TGL, and HDL.

6. Lipids and Apo B Levels in the Study Population:

Table showing fasting plasma lipids and lipoproteins in CAD – and CAD + patients as assessed by angiography.

	CAD +	CAD -	p value (t – test)
Apo B	1.10 ± 0.40	0.76 ± 0.18	<0.0001
TC	171.61 ± 60.58	157.78 ± 35.72	<0.05
TGL	197.16 ± 167.27	145.50 ± 70.79	<0.005
HDL	40.85 ± 11.66	43.12 ± 11.09	<0.16
LDL	94.42 ± 50.14	88.07 ± 28.56	<0.27
Age	55.54 ± 8.77	51.06 ± 8.79	<0.0001

Fasting plasma concentrations of TC, TGL and Apo B were significantly higher in CAD + patients than in CAD – patients. CAD + patients were significantly older than CAD – patients.

7. Lipids and Apo B in patients on Statins:

Table showing Fasting lipids and apolipoprotein B in patients on statins classified as CAD + and CAD -.

	CAD + (numbers)	CAD – (numbers)	p value (Chi square test)
TC > 200mg/dl	14	5	< 1
TGL>150mg/dl	35	16	< 0.20
HDL>40mg/dl	30	21	< 1
LDL>130mg/dl	9	2	< 0.20
Apo B>0.99g/l	44	4	< 0.001

In patients on statins only apo B, but not LDL was able to predict the presence of underlying CAD.

8. Logistic Regression Analysis:

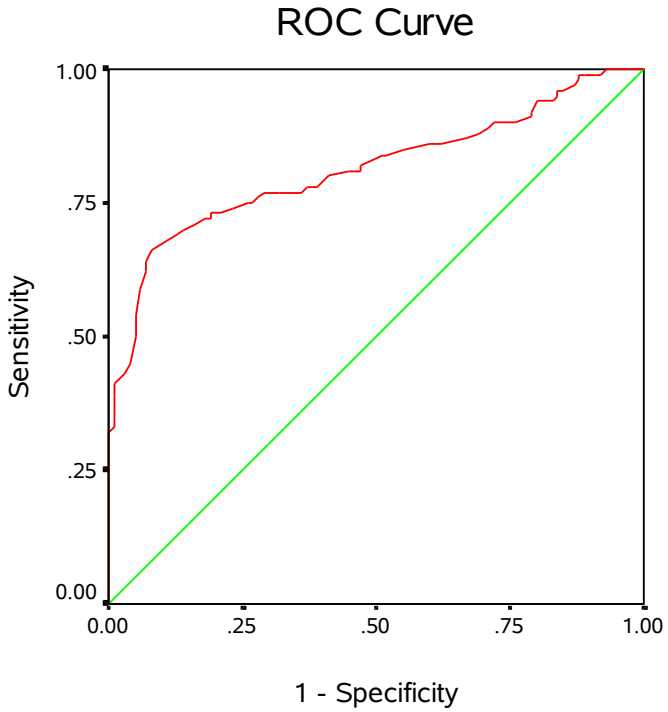
In multivariate analysis using binary logistic regression only age, HDL and Apo B emerged to be significantly associated with the presence of underlying CAD.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	TC	-.035	.023	2.256	1	.133	.966
	TGL	.001	.004	.032	1	.857	1.001
	HDL	.065	.030	4.669	1	.031	1.067
	LDL	.028	.022	1.557	1	.212	1.028
	AGE	-.062	.018	12.227	1	.000	.940
	Constant	3.576	1.222	8.569	1	.003	35.729

a. Variable(s) entered on step 1: TC, TGL, HDL, LDL, AGE.

9. ROC curve (Apo B):



ROC analysis revealed that a cut off value of apo B more than or equal to 0.99 g/l has 66% sensitivity and 92% specificity for detection of underlying CAD.

10. Among patients with CAD - 63.86% of patients with normal LDL levels had apo B values more than 0.99 g/l.

DISCUSSION

Apo B has emerged to be a novel marker of risk in patients with cardiovascular disease. Apo B serves as a predictor of future cardiovascular events. Atherosclerosis is a complex series of biological responses to the trapping of an atherogenic particle within the arterial wall, not simply a piling up of cholesterol. Injury to the endothelium, oxidation of the apo B and phospholipids as well as the cholesterol within the LDL particle, and uptake of LDL by macrophages all trigger a wide, intricate, and damaging series of inflammatory and healing responses. Each atherogenic particle contains 1 molecule of apo B, and therefore plasma apo B represents the total atherogenic particle number[29]. In this study we report the association between plasma apo B and CAD in patients who were admitted for elective coronary angiography.

The most prevalent among the traditional risk factors were hypertension, diabetes mellitus & smoking which are well known to be strong coronary risk factors. Among the traditional risk factors age, gender, smoking, total cholesterol (TC), TGL, LDL were found to be significantly correlated with CAD. The median apo B level among patients with CAD was 1.07g/L. The median value of apo B was higher in patients with CAD compared to those without CAD. Apo B was superior to the traditional lipids - TC, LDL, HDL, and TGL in predicting the presence of CAD.

Among patients on statins, only apo B was able to predict the presence of CAD. Even in the patient population with CAD and normal LDL levels, apo B was able to predict the presence of CAD.

The role of apo B as an important risk factor is biologically plausible,[60, 61] since plasma apo B concentrations reflect the number of atherogenic lipoprotein particles.[62] The atherogenic lipoproteins are LDL, containing predominantly cholesterol, VLDL remnants (IDL), and chylomicron remnants, which contain both cholesterol and TGL. LDL and remnant particles each contain 1 molecule of apo B as the structural protein, whereas the amount of cholesterol and TGL per particle varies, and with it, the atherogenicity of the particle. Large, Triglyceride (TG)-rich VLDL particles are not considered atherogenic,[63] whereas smaller remnants of TG-rich lipoprotein particles are atherogenic. [64] Small, dense LDL particles are more atherogenic than are LDL particles of normal composition.[65] Increased small, dense LDL concentrations are reflected by a more pronounced elevation of LDL apo B than of LDL-chol, and this trend is often accompanied by elevated plasma TG concentrations. Total Cholesterol is a constituent of both atherogenic, apo B containing lipoproteins and antiatherogenic, apo A-containing lipoproteins. This heterogeneity of lipoprotein particle composition can explain the superiority of apo B over TC and TGL as a CAD risk factor.

In our study apo B was the best discriminant between CAD + and non-CAD patients.

Other studies have shown that the predictive power of apo B can be increased by separation of the plasma into different apo B containing lipoprotein fractions. The fasting remnant, or IDL fraction, was superior to the LDL fraction in predicting the presence[66] or severity[67] of CAD in women with premature CAD. Postprandial chylomicron remnant concentrations were higher in CAD+ than in CAD- patients while LDL apo B was similar.[68] IDL-cholesterol was also related to the progression of CAD in a combined group of men and women (n=63) with premature CAD. [69] Thus, parameters for remanant particles are potentially better CAD risk indicators than is plasma apo B.

Our results suggest that apo B is a better predictor of CAD than traditional lipid profile especially on patients with statin treatment. To the best of our knowledge this is the first Indian study to show the same. Higher apo B values were noted in patients with CAD and normal levels of LDL – C as well. Thus apo B is a better marker than routine lipid profile to be used both in general as well as CAD population.

LIMITATIONS

1. The numbers of patients studied were small and so the study was not adequately powered to pick up certain associations. The fact that established risk factors like diabetes mellitus was not significantly related to CAD could have been partly related to small numbers.

2. Quantification of coronary angiographic findings was limited to the visual interpretation, though this is representative of “real world” practice.

3. Only patients with clinically suspected CAD were included in the study. Hence the cut off value of apo B as predicted by ROC analysis may not be applicable for the general population.

4. Eventhough coronary angiography was analysed in the present study, the outcomes of adverse cardiovascular events and mortality were not studied.

5. Our controls were selected from treadmill negative group. Treadmill testing cannot establish reliably that these patients had normal coronary angiogram.

CONCLUSION

1. Apolipoprotein B levels provide better information regarding the presence of Coronary Artery Disease.

2. Higher apo B values were noted even in those patients with CAD with normal levels of LDL Cholesterol.

3. In patients who were on statins, only apo B was able to predict the presence of Coronary Artery Disease.

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ABBREVATIONS AND ACRONYMS

Apo B	-	Apolipoprotein B
Apo [a]	-	Apolipoprotein A
Apo C	-	Apolipoprotein C
CAD	-	Coronary Artery Disease
CHD	-	Coronary Heart Disease
IHD	-	Ischemic Heart Disease
LDL	-	Low Density Lipoprotein
HDL	-	High Density Lipoprotein
TGL	-	Triglycerides
TC	-	Total Cholesterol
VLDL	-	Very Low Density Lipoprotein
IDL	-	Intermediate Density Lipoprotein
CAG	-	Coronary Angiography
KDa	-	KiloDaltons
TGRL	-	Triglyceride Rich Lipoproteins
SVD	-	Single Vessel Disease
DVD	-	Double Vessel Disease
TVD	-	Triple Vessel Disease
S.NO	-	Serial Number

HOSP. NO	-	Hospital Number
TMT	-	TreadMill Test
DM	-	Diabetes Mellitus
H	-	Hypertension
S	-	Smoking
DL	-	Dyslipidemia
F	-	Family History of CAD
M	-	Old Myocardial Infarction
PO	-	Positive
NE	-	Negative
NO	-	Not Done
INCO	-	Inconclusive
M	-	Male
F	-	Female
S	-	Single vessel disease
D	-	Double vessel disease
T	-	Triple vessel disease
M	-	Minor coronary artery Disease
N	-	Normal coronaries

PROFORMA

S.No:

Hosp. No.

Name

Age :

Sex :M / F

Address :

Typical chest pain: Class I / II / III / IV / No

Dyspnea: Class I / II / III / IV / No

Other symptoms:

Diabetes Mellitus 1. Yes 2. No

Hypertension 1. Yes 2. No

Smoking 1. Yes 2. No

Dyslipidemia 1. Yes 2. No

Family h/o of IHD 1. Yes 2. No

Past h/o of MI 1. Yes 2. No

Lipids: TC: TGL: HDL: LDL:

TMT: Positive / Negative / Inconclusive / No

CAD: SVD / DVD / TVD / Minor / No

APO – B level:

STUDY DATA

S.No	Name	H.NO	APO - B	Age	Sex	CAD	TC	TGL	HDL	LDL	TMT	Risk factors	Statins
1	Minati Sen	447040C	0.65	57	F	D	188	111	59	107	NO	DM	YES
2	Lila Karna	455104C	0.46	49	F	N	140	94	50	71	NE	DM	NO
3	Naresh Kumar	778887B	0.71	61	M	S	161	202	27	94	NE	DM, H, S, DL, F	YES
4	Aijt Kumar Saha	422157C	0.96	55	M	S	162	182	43	83	PO	DM, H, DL	YES
5	Ratan Kumar	455789C	1.02	52	M	D	141	102	34	87	PO	S	NO
6	Subramanian. K	910228A	0.86	58	M	T	113	100	38	55	PO	H, DL, F, M	YES
7	Prabhat Nalini	081726A	0.94	47	F	N	161	148	40	91	NO	DM, H	NO
8	Subash Saha	456784C	1.05	44	M	T	116	113	35	58	PO	F, M, S	YES
9	Muthusamy . P	453112C	1.2	57	M	T	141	131	31	84	PO	S, M	YES
10	Matur Rahman	456392C	1.54	54	M	T	114	360	32	10	NO	DM, H, FH, M	YES
11	Gita Mukerjee	457222C	0.84	54	F	N	185	199	40	105	PO	D	NO
12	Ramaswamy.	001538C	0.83	66	M	T	203	154	36	136	PO	H,M	NO
13	Shyam Sundar	451124C	1.85	45	M	T	146	289	39	51	PO	DM,S,DL,M	YES
14	Pravin Chandra	549534B	0.74	56	M	T	202	119	43	135	PO	H,S,M	NO
15	Chunilal Kanjilal	455251C	0.8	48	M	N	127	156	35	61	PO	H,S	YES
16	Saroj Samantha	514805C	0.71	68	M	N	145	56	64	70	NO	N	NO
17	Gopi Mohan Paul	456310C	0.99	57	M	M	147	195	28	80	NO	H,S	YES
18	Mani	457752C	2	61	M	S	147	178	36	75	PO	H	YES
19	Shankar Sharma	458139C	2.41	50	M	S	137	132	37	74	NE	H,S	YES
20	Swapan Chakrabortty	456865C	0.59	49	M	T	214	151	52	132	PO	DM,S,DL	NO
21	Francis D'costa	457416C	0.81	67	M	T	167	182	33	98	NO	H	NO
22	Vimal Kejriwal	456044C	1.03	52	M	D	123	171	33	56	NE	S	NO
23	Annamma Varghese	458525C	0.9	54	F	N	248	110	58	168	NE	DM,H,DL,FH	NO
24	Kulanthai Raj	518288C	0.85	48	M	N	169	214	62	64	NO	H,DL	NO
25	Niranjan Sadhukhan	458478C	0.87	68	M	D	124	216	32	49	NO	DM,H,F,M	YES
26	Bholanath Pandey	457126C	1.09	59	M	S	157	82	45	96	NE	M	YES
27	Kalawati Mandal	457753C	0.84	47	F	N	161	148	48	83	INCON	DM,H	NO
28	Rahima Bee	739344A	0.74	48	F	M	188	95	41	128	NE	DM,H,DL,F,M	YES
29	Naranath Hyderaly	454478C	1.03	61	M	M	205	160	126	47	PO	N	YES
30	Biswadeb Sarkar	458488C	0.99	42	M	N	185	133	116	92	PO	S	NO
31	Joseph Xavier	449631C	0.7	52	M	D	123	105	40	62	NO	S	YES
32	Embrose	042582A	1.09	53	M	T	159	293	39	61	NO	DM,F	YES
33	Madan Mohan	448575C	0.56	64	M	D	113	73	44	54	NO	N	NO
34	Pradip Kumar Garai	459099C	0.93	48	M	T	182	221	36	102	NO	H	NO
35	Jeevanandan.M	286507C	0.62	54	M	N	120	126	39	56	PO	H	NO
36	Bijay Kanta Dey	428335C	1.08	65	M	S	166	123	50	91	PO	DM,H,S	NO
37	Rabindra Kumar Dash	143683C	0.41	59	M	M	213	118	39	150	NE	H,S,DL	NO
38	Ram Chandra Das	460168C	1.2	49	M	D	127	116	41	63	NE	S,F	YES
39	Maya Saha	925717B	1.14	56	F	D	151	142	35	68	PO	H,DL	YES
40	Ramendra Gopal Sen	138117C	1.45	58	M	D	170	90	49	102	NO	N	NO
41	Sujit Sarkar	457721C	1.05	49	M	T	120	206	49	30	PO	H,S,DL,F,M	YES
42	Brajbahari Sharma	936034B	0.99	57	M	T	131	226	35	51	PO	DM,H,DL,M	YES
43	Prahlad Chandra Sark	459645C	0.96	45	M	N	181	287	48	76	PO	DM,H,DL	NO
44	Sisir Kumar Nayak	441710C	1.02	49	M	D	140	120	39	77	PO	DM,S,DL,M	NO

45	A.Aandi	158702B	0.89	62	M	T	136	81	37	83	PO	H,S,DL	YES
46	Pradyut Kumar Das	460247C	0.8	56	M	S	131	145	35	67	NO	DL	YES
47	Rameshwar	442317C	0.9	43	M	D	112	119	34	54	NO	H,DL	NO
48	Prawn Krishna	503067C	0.78	59	M	T	226	84	65	144	NO	DM,H,S,DL,F,M	NO
49	Abdul Mumith	459773C	1.3	55	M	D	183	120	39	120	PO	DM,S,DL,M	NO
50	Lalchuni Devi	391545C	0.49	63	F	M	145	140	51	66	NO	DM,H,DL	YES
51	Swarna Kumari	419682C	0.62	44	F	N	204	53	58	135	NO	N	NO
52	Sreenivas Rao	432371C	0.68	67	M	T	170	119	35	111	NO	DM,H,DL	NO
53	Nirmal Sen Gupta	454759C	0.69	62	M	D	178	108	37	119	NO	DM,H,DL	YES
54	Lawan Kumar	455133C	0.75	50	M	D	101	79	39	46	PO	DM,H	YES
55	Kalidas Chowdhury	454717C	0.84	56	M	M	189	350	47	72	INCON	S,DL	YES
56	Hem Borkakoty	393859C	0.66	49	M	M	88	106	24	43	NO	DM,H	NO
57	Shis Pal Singh	453791C	1.2	32	M	S	134	93	30	85	NO	M	NO
58	Byomkesh Das	359814C	1.03	62	M	S	237	549	48	79	NO	H,S	NO
59	Rajammal	702081A	1.31	65	F	T	211	109	46	143	PO	DM,H,DL	NO
60	Lakshmipathy. A	440821C	1.04	53	M	T	249	264	49	147	PO	DM	NO
61	Raj Kishore Prasad	357185C	0.77	55	M	D	153	149	39	84	NE	H,S,DL	NO
62	Samuel	*004490	0.61	73	M	T	125	111	35	68	PO	DM,H,DL,F	YES
63	Basanthi Sinha	465133C	0.54	53	F	N	153	117	49	87	PO	H	NO
64	subramaniam.v	473315C	1.67	48	M	D	137	125	38	74	PO	DM,H,S	YES
65	Arumugam	473030C	2.5	24	M	T	263	312	41	160	NO	DM,H,S,DL,M	YES
66	Sivagnanam.V.K	462335C	0.98	71	M	T	123	199	35	48	NO	H,DL	NO
67	Thansangh	678226C	0.71	58	M	N	177	160	64	44	NE	DM,H	NO
68	Shankar Lal	552638C	0.8	57	M	M	178	148	52	96	NO	S	NO
69	Shyamala sarkar	482138C	0.64	42	F	T	162	169	42	86	PO	H,M	YES
70	Nagarajan ramdoss	422882C	0.55	52	M	D	168	277	48	65	NO	H	YES
71	Roshan Ali	677567C	0.79	48	M	N	147	124	37	94	NE	F	YES
72	shir shir kumar	486663C	1.3	55	M	T	195	239	36	111	NO	H,S,DL	NO
73	Shet Sonwani	489238C	1.06	52	M	S	102	248	15	37	PO	DM,H,S,DL	YES
74	Munirathinam	093536A	0.63	73	M	S	127	73	37	75	NO	DM, S, M	YES
75	Jeremiah	205001	0.97	67	M	S	153	120	38	91	NO	DM,H	NO
76	Gordai Bauri	680071C	0.86	50	M	N	151	134	43	93	NE	H,S,DL,F,M	YES
77	Ram Nandan Singh	228177B	0.51	71	M	D	103	64	42	48	NO	N	YES
78	Muthusamy.R	029907C	1.3	60	M	M	164	79	56	92	NO	H	YES
79	Janardan Karmakar	551263C	1.61	51	M	T	242	240	44	150	NO	H,S	YES
80	Hari Barman	513788C	0.8	37	M	N	211	116	58	130	NO	F	NO
81	Renu Bala	533033C	0.57	64	F	T	105	130	39	46	NO	DM,H,F	NO
82	Sachin Kumar Hazra	546860C	0.98	47	M	N	195	219	48	104	NE	H,S,M	YES
83	Alok Ghosh	660765B	0.57	48	M	N	185	122	49	112	NO	H	NO
84	Chandra Pon Mudhi	506621C	1.13	55	F	N	206	197	54	113	PO	DM,H,DL,F	YES
85	Karimunisse	722359A	1.36	47	F	D	197	218	45	108	PO	DM,DL	NO
86	Umeshwar	503773C	1.01	50	M	T	134	174	45	54	PO	DM,S,DL,F	NO
87	Rabindra Nath	548632C	1.04	51	M	M	195	145	35	125	PO	H,S	YES
88	Kisun Viswa Karma	549030C	0.66	58	M	M	123	157	34	58	NO	DM,H,S,DL	YES
89	Alli Rani	119662	0.62	36	F	N	172	99	44	108	NE	N	NO
90	Durgapada Roy	558764C	1.05	52	M	S	168	103	46	101	NO	S,F,M	YES
91	Shaik Zahiruddin	793672B	1.01	48	M	T	70	123	17	28	NO	H,S,M	NO
92	Narayan Singh	236874C	0.63	62	M	T	92	110	28	42	NO	H,S,DL	YES
93	Ramjay Rawani	523612C	1.12	55	M	S	153	139	42	83	PO	DM,H,S	YES
94	Budhu Lal Dey	492137C	0.91	46	M	N	151	235	42	62	NO	H,S,DL	NO

95	Naresh Prasad Yadav	474418C	0.61	56	M	N	177	178	41	100	NO	DM,H,S,M	YES
96	Ashok Chowdhury	539301C	0.49	63	M	M	123	122	36	63	NO	H,S	YES
97	Fulmati Devi	440404C	0.87	53	F	N	176	162	44	100	NO	DM,H	NO
98	Nand Kishore Thakur	535549C	1.18	58	M	S	120	225	45	30	NE	DM	YES
99	Sujatha Balachandran	090141A	1.08	51	F	S	164	148	41	93	NE	S	YES
100	Shankar	529193C	0.65	58	M	N	85	100	35	50	NE	N	YES
101	Unnithan	510651C	0.74	56	M	N	173	128	42	105	NO	H	NO
102	Randhir Prasad	477823C	1.58	69	M	D	152	79	54	82	NO	H	YES
103	Shruti Devi	563895C	0.95	53	M	D	230	410	43	105	NO	DM,H,M	YES
104	Sudha Ranjan	571009C	1.53	64	M	M	124	94	35	70	NE	H,S	NO
105	Rama Ranjan	002485C	0.89	39	M	N	80	77	28	37	NO	S	NO
106	Ghana Shyam	578308C	1.05	40	M	T	172	195	42	91	NE	DM,H	YES
107	Jacob. P. I	572268C	1.04	45	M	D	202	67	39	150	PO	H,M	NO
108	Ekambaram	339092B	1.2	65	M	T	146	182	30	80	NO	H,S,M	NO
109	Venkatesaperumal	655938A	1.3	65	M	D	248	255	58	139	NO	DM,H	YES
110	Maya Thakur	591011C	0.89	55	F	N	211	234	49	156	NO	H	NO
111	Sundaresan	701287A	0.83	78	M	M	118	81	30	72	NO	S	YES
112	Sumati Ghosh	591993C	0.7	55	F	N	182	139	42	112	PO	H	YES
113	Shahajan Begam	614037C	0.71	43	F	N	138	123	35	81	NO	N	NO
114	Ranu Sengupta	603686C	0.63	50	F	N	139	127	49	73	NO	N	NO
115	Moni Ghosh	610159C	0.71	39	F	M	180	139	47	105	NO	DM, H, DL	YES
116	Nisith	052748c	0.92	63	M	N	126	156	41	54	NE	H, S, DL	YES
117	Subash Chandra	370534C	1.11	65	M	S	194	237	48	118	NO	DM, H, DL	YES
118	Dilip Kumar	615714C	1.11	49	M	T	206	276	50	110	NO	DM, H, DL	NO
119	Ashish Kumar	675369A	0.76	43	M	N	134	94	41	74	NO	N	NO
120	Rita Devi	530820C	0.85	51	F	M	162	187	43	86	NO	DM, DL	YES
121	Ramani Mohan Debnath	616382C	0.95	75	M	N	179	215	48	104	NO	H, S	NO
122	Tapas Kumar.B	618545C	0.84	48	M	N	136	120	36	83	NO	S	NO
123	Mohanam	587470C	1.21	55	M	T	566	381	55	435	NO	DM, DL	YES
124	Shankar Singh	529183C	0.65	56	M	N	88	103	33	49	NO	DM, S, H, DL	YES
125	James Thilagar	562664C	2.01	53	M	D	180	285	43	92	NO	DM, DL	YES
126	Ran Sebak Shaw	949773B	1.06	53	M	T	196	447	40	67	PO	H, DM, S, FH, DL	NO
127	Azeez Khan .M	557463C	0.73	46	M	N	113	205	30	53	NO	S, DL	YES
128	Brajanath Dutta	618090C	1.02	52	M	D	178	231	41	98	NO	DM, H, DL, ES	YES
129	Ghana Voli	262613	1.04	60	M	T	305	900	50	82	NO	DM, DL	YES
130	ShivaKumar	591297C	1.2	56	M	T	196	243	40	118	NO	H, S	YES
131	Bichitra Das	620512C	1.03	40	M	T	113	85	33	59	PO	N	YES
132	Tamil Selvi	667381A	0.77	48	F	N	155	80	36	99	NO	FH	NO
133	Achuthan	613903C	0.74	55	M	M	151	149	34	94	NO	H, S	YES
134	Parvatheiswar Rao	606892C	0.82	63	M	M	187	389	38	71	NO	DM, H	NO
135	Saradindu Naaya	622401C	0.68	55	M	M	160	200	43	73	PO	N	NO
136	Yesupatham	125919B	1.06	62	M	N	143	246	32	80	NO	DM	YES
137	Anjali Kamila	625503C	1.24	47	F	S	211	219	45	112	NE	N	YES
138	Subba Lakshmi	049653B	1.23	59	F	T	202	213	40	136	NO	DM, H, DL	YES
139	Susti Rani	626159C	1	57	F	N	186	163	35	121	NO	H	NO
140	Subrata Chakraborty	486159C	1.28	53	M	T	241	348	44	145	PO	ES	NO
141	Kailash Pati	629545C	1.43	60	M	T	263	114	43	217	PO	H, FH	YES
142	Prabir Lal Banerjee	629315C	0.67	53	M	N	115	79	41	59	NO	N	YES
143	Dasan Gnanaraj	478048C	1.13	59	M	T	177	172	32	111	NO	DM, ES, DL	YES
144	TapanKumar Bhuni	629623C	0.53	39	M	N	139	137	55	64	NO	FH	YES

145 Padmanaban	613965C	0.74	57 M	M	192	193	36	123 NO	DM, H	YES
146 Rathindra Nath Das	629537C	1.36	45 M	S	245	131	39	196 NO	N	YES
147 Praveer Kumar	619308C	0.92	44 M	N	104	54	31	60 NO	DM, H	NO
148 Rabindranath Dutta	631981C	1.09	55 M	S	165	172	36	99 NO	H, S	NO
149 Chandra Kumar	630899C	1.28	58 M	M	247	258	45	161 NO	DM, H, ES	YES
150 Parvathi	277063B	0.72	55 F	N	138	202	39	62 NO	DM, H	YES
151 Bipad Bavan	634926C	1.03	57 M	S	174	249	35	94 NO	DM, S	YES
152 Saresh Biswas	632234C	1.07	59 M	S	209	193	41	124 NO	N	NO
153 Aloke	646527C	0.77	49 M	S	138	141	31	86 NE	ES, H, DL	YES
154 Jerry William	636825C	0.81	35 M	S	142	130	36	101 NE	DM	NO
155 Dipti Rani	663255C	0.8	62 F	N	224	148	59	150 NE	H, S	YES
156 Ajit	692173c	0.82	52 M	N	184	247	35	107 NE	S	NO
157 Kavalagam	656248C	0.95	67 M	N	158	129	33	102 NE	S	NO
158 Swapan Kumar	663122C	0.69	45 M	N	118	152	34	57 NE	S	NO
159 Nirmal	642857C	0.96	46 M	N	235	520	50	103 NE	DM, H	YES
160 Sabitha Chaterjee	930563A	0.75	47 M	N	162	72	49	90 NE	H, DL	NO
161 Raja Kumari	629835C	0.89	55 F	N	164	80	55	99 NE	DM	NO
162 Maidul Islam	664214C	0.53	61 M	N	104	144	34	41 NE	ES, H	YES
163 Md Azad	718987B	0.51	52 M	N	87	94	27	42 NE	DM,H	YES
164 Girija	665973C	0.46	55 F	N	122	62	50	48 NE	DM	YES
165 Mahendra	593556C	0.66	48 M	N	113	71	31	68 NE	ES	NO
166 Chhaya	663886C	0.77	51 F	N	130	111	32	77 NE	N	YES
167 Sabitha cihish	665593C	1.05	64 F	S	159	318	38	70 NE	H	YES
168 Bhavati roy	675395c	1.05	46 F	N	184	150	47	103 NE	H, FH	YES
169 Maya rani ghosh	675745c	1.03	50 F	N	194	184	45	115 NE	N	NO
170 Prabhot	675530c	0.75	62 M	N	164	86	49	93 NE	DM	NO
171 pandian	646209c	0.99	40 M	N	181	204	46	102 NE	S, DM	YES
172 Gobinda	674311c	0.81	49 M	N	136	167	33	67 NE	DM	NO
173 Jiban	676789c	0.81	49 M	N	142	156	38	90 NE	FH	NO
174 Habibor	676673c	0.71	39 M	N	151	147	42	88 NE	N	NO
175 Pranab	676647c	1.02	53 M	N	198	159	48	122 NE	S	NO
176 Manju	677075c	0.56	47 F	N	113	64	42	58 NE	DM	NO
177 Paritosh	593070c	0.65	42 M	N	130	72	35	84 NE	DM	NO
178 Bidhan	677046c	0.98	41 M	N	181	135	44	113 NE	N	NO
179 Anant	677121c	0.77	57 M	N	170	100	50	87 NE	FH	NO
180 Akhtaruzzaman	675890C	0.85	38 M	N	177	143	32	109 NE	S	YES
181 Antony	311548b	0.97	60 M	N	184	178	53	93 NE	DM, H	NO
182 Md. Amiruddin	675341c	0.88	45 M	N	166	141	40	91 NE	N	NO
183 Shyama Kumar	676198c	1.07	42 M	N	171	265	36	84 NE	H	NO
184 Asha	677077c	0.77	45 F	N	166	79	48	103 NE	N	NO
185 Chanchalundu	677712C	0.79	63 M	N	170	93	48	91 NE	S, H	YES
186 Tapan Kumar	678011C	0.75	51 M	N	166	110	48	83 NE	N	YES
187 Animesh	588790c	1.07	58 M	N	211	177	53	123 NE	DM	YES
188 Muzubur	677639C	0.99	33 M	N	166	88	39	110 NE	DM	NO
189 Nirpendra nath	679067c	0.11	56 M	N	179	97	48	103 NE	H	NO
190 Chhabi Rani	679741c	0.33	47 F	N	88	60	48	27 NE	N	NO
191 Rajadurai. A	678511c	0.57	48 M	N	97	107	29	44 NE	S	YES
192 Saraswati	557176C	3	51 F	D	283	1409	34	98 NO	DM	YES
193 Anil Kumar	451632C	1	63 M	T	154	155	48	91 NO	N	YES
194 Kalipada Saha	614722C	1	74 M	S	211	151	46	142 NO	N	YES

195 Golak	120175C	1.07	55	M	S	159	141	33	98 NO	ES	YES
196 Raja	672430B	0.52	37	M	M	222	164	36	153 NO	DM,H,S,M	YES
197 InderJit Singh	569591C	0.74	38	M	N	86	142	36	27 PO	H,DL	NO
198 Dipankar	066287c	0.71	37	M	N	155	115	43	99 NE	ES	NO
199 Susheela	776812b	0.65	61	F	N	132	71	40	82 NE	N	NO
200 Bijoy Kumar	694144c	0.7	55	F	N	130	160	36	67 NE	H	NO